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Stability studies of nano-cream containing piroxicam

Muthanna F. Abdulkarim[1*], Ghassan Zuhair Abdullah[1*], Mallikarjun Chitneni[1], Elrashid Saleh Mahdi[1], Mun Fei Yam[1,2], Ahmed Faisal[1], Ibrahim M. Salman[1], Omar Ziad Ameer[1], Mohanad N Sahib[1], Munavvar Zubaid Abdulsattar[1], Mahiran Basri[3], Azmin Mohd Noor[1]

***Corresponding author:**

Muthanna F. Abdulkarim
[1]School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Minden, Pulau Penang, Malaysia.
[2]Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia.
Tel: +60164849551
Email: muthana_albuldawy@yahoo.com

Abstract

The aim of this study is to study the stability of the nano-cream formula containing the newly synthesized palm oil esters when stored for reasonable storage duration. The prepared 0.5% piroxicam nano-cream formula contained phosphate buffer as external phase, palm oil esters as the oil phase and a combination of (80:20) of Tween 80 and Span 20 as emulsifier at a ratio of 37:25:38, respectively. Piroxicam is a hydro-lipophobic drug. Stability on storage is an important aspect which ensures the dosage form can exert the effects it is supposed to exert for the duration of storage. Droplets size, electrical conductivity, drug content, pH and rheological parameters are the parameters that have been assessed under different temperature to evaluate the stability of nano-cream preparation. Thus, experiments which measure the above parameters were conducted at storage temperatures of 4, 25 and 40^{o}C.The data obtained from the stability study conducted on nano-cream formula showed that this formulation was stable for the whole 3 months period of the study when stored at tested several temperatures.

Keywords: Palm oil esters; Nonionic surfactant; Piroxicam; Nano-cream

Introduction

In general, the purpose of stability testing is to provide evidence of how the quality of drug product varies with time under the influence of some environmental factors like temperature, humidity and light that leading to set the shelf-life of pharmaceutical product. Since emulsions are biphasic systems, they are inherently unstable according to the second law of thermodynamics. The degree and speed of destabilization vary from system to system. So, it is important for a formulator to carry out studies on the stability of the system. Emulsions in general can be destabilized by the following mechanisms: Creaming, flocculation [1], Coalescence [2] and Ostwald ripening [3].

Creaming is the separation of emulsion phases based on the density difference between the dispersed phase and the dispersion medium. Creaming is an undesirable process; however, it is a reversible process. To prevent creaming, the density of dispersed phase and the dispersion medium should be fairly even. The other factors which govern creaming according to stokes law are the dispersed phase globule size and the viscosity of the external phase. Flocculation is the reversible associations of the dispersed droplets by certain weak bonds that can be easily redisperse through vigorous shaking. Flocculated droplets are characterised by their ability to maintain their shapes and sizes.

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Flocculation may lead to irreversible coalescence. In systems stabilized by non-ionic surfactants, the dispersed phase droplets are attracted to each other by van der waals forces, but prevented from coalescence by repulsion due to steric hinderance [4]. The van der walls attractive force between the droplets is dependant on radii of the dispersed phase droplets. As the radius of the droplet decreases, the potential attraction also decreases [5]. As the concentration of surfactant increases in emulsion, there will also be an increase in thickness of the film formed around the droplets by the surfactant molecules which leads to an increase in steric repulsion, thereby rendering the emulsion to be more stable. So, flocculation may not be an important parameter to be studied in case of micro and nano-emulsions as the particle size is very small and the concentrations of surfactant present in them is high.

On the other hand, coalescence is the irreversible combination of two droplets to form a larger droplet. Similarly, Ostwald ripening is the irreversible transfer of small droplets into larger ones so as to form new droplets. Ostwald ripening phenomenon is concerned with systems in which high variation in the droplets sizes is found. Ostwald ripening is the major concern of the stability issue to be addressed by formulators for a successful micro or nano-emulsion system. Coalescence and/or Ostwald ripening phenomena lead finally to separation of these systems into three phases - the internal, external and the emulsifier phase. Accordingly, there is another form of emulsion instability which is the phase inversion of dispersed system. This is characterised by the transformation of the internal phase into the external phase and reversely the external phase to the internal phase.

Stability studies under normal storage conditions can be efficient in predicting the stability of the system, however, the time involved is an obstacle. In order to generate stability data fast and reliably, accelerated stability studies are carried out. Measuring of physicochemical properties of the product under accelerated conditions can reflect the performance of the product over time. Chemically, a change in pH of the formulation can indicate a degradation or ionization of one or more of the ingredients in the formula. Furthermore, chemical transformation of ingredients reflects their incompatibility or degradation which in turn can produce chemically toxic effect on consumers. Besides, conductivity is one of the techniques that is used to identify the type of emulsion if it is W/O or O/W. Presence of water in the external phase results in a conductive system. Any inversion in the system can be identified by measurement of formula´s conductivity. The measurement of conductivity at different parts of the emulsion container would be an advanced detection of creaming or sedimentation [6]. Droplet size measurement is a very important factor in assessing the stability of the emulsion system. Changing of the droplet size with time is the direct result of the aggregation and combination of the internal phase droplets to form larger droplets [6]. Viscosity of the system is of high importance in the formation and continuation of the emulsion. Relatively, reduction of the viscosity with time can indicate a kinetically unstable emulsion where the free moveable droplets collide with each other and tend to coalesce. Hence, detection of viscosity changing with time can provide data about system stability.

The stability of the emulsion is concerned with the maintenance of internal phase dispersion in the external phase without having effective changes for both of them. In other words, the system should maintain the same number and sizes of the dispersed droplets in the dispersion media. Piroxicam is a water insoluble drug with an acidic pKa value of 5.3 [7]. It has low solubility in water and in oil as well. Hence, the formulation of piroxicam as O/W nano-cream is supposed to be responsible of instability of the system. Therefore, stability of this system is critical factor that is needed to be assessed.

Materials and methods

Materials

Disodium hydrogen phosphate was supplied by R & M Chemicals (UK). Orthophosphoric acid, hydrochloric acid and glacial acetic acid were supplied by BDH (UK). Methanol and acetonitrile (HPLC grade) were purchased from J.T. Baker (USA). Nylon membrane filters (0.45 µm pore size) was purchased from Whatman International (UK). Potassium dihydrogen phosphate and sodium hydroxide were supplied by R & M chemicals (UK). POEs was a gift from UPM.

Methods

Method of preparation of selected formulation

Formulae E14 to E16 were prepared according to Continental method. Oil (POEs) and surfactant mixture were mixed thoroughly for 15 minutes at 750 rpm in a beaker by using low shear mixer fixed with three blades (propeller). Piroxicam was added to the mixture and mixed for another 30 minutes until dissolved completely. Aqueous external phase (phosphate buffer of specified pH) containing sodium benzoate (0.01%) was added gradually to the above mixture and mixed for another 30 minutes.

Before carrying out the stability study for a period of three months, the tested formulations of E14, E15 and E16 were subjected to precipitation test under accelerated condition of 40^{o}C for 15 days. The formulations which showed no drug precipitation were then subjected to stability tests for a period of three months at three different temperatures. Accelerated stability studies were performed by placing the samples in the oven at 40^{o}C. On the other hand, the intermediate stability study was conducted at 25^{o}C temperature by placing the samples at room temperature. The stability study was also carried out at 4^{o}C by placing the sample in the refrigerator. At periodic intervals of 15, 30, 45, 60 and 90 days, all of the samples which were stored at 4, 25 and 40^{o}C were studied for the conductivity, pH, droplet size and rheological properties and were compared to the initial values of respective measurements. All the measurements were carried out in triplicates.

Droplet size measurement

The droplet size of formulation E16 was measured by using Malvern zeta sizer 1000 HAS, Malvern Works, UK which is based on the basic principle of photon correlation microscopy. The limit of detection of these equipments ranged between 1 nm to 1 micrometer. The samples were diluted with the corresponding buffer of the external phase to get the K count in between 50 -200 as required by machine consistency before reading the droplets size. The mean droplet size of the freshly prepared formulation was measured and was used for the purpose of comparing the change in the mean droplet size of the formulation after specified time intervals at different temperatures studied.

Conductivity measurement

The conductivity of formulation E16 was measured by using the conductometer (Cyberscan, Singapore, eutech instrument). An amount of 2 gm of the sample was transferred into a beaker and the conductometer probe was immersed into the bottom of the container. Then the conductivity reading in µs was recorded. Conductivity of freshly prepared formulations was measured and was used to compare the change in conductivity of the formulation after specified time intervals at different temperatures studied.

pH measurement

Measurement of pH of the samples was made by using the pH meter (cyberscan, eutech instrument). Two grams of the sample was transferred into a beaker and the pH meter probe was immersed into the container. Then the pH reading was recorded. The pH meter was calibrated before using it to measure the pH of the nano-cream. The pH of the freshly prepared formulation was measured and was used to compare the change in pH of the formulation after specified time intervals at different temperatures studied.

Intrinsic viscosity measurements

Intrinsic viscosity of formulation E16 was measured by using a rheometer (rheologica instrument AB, Sweden). The system was equipped with a cone and plate measuring head (plate diameter 40 mm). About 0.5 gm of the sample to be analysed was placed on the plate and left to equilibrate with the controlled temperature (25°C±0.1) for 3 minutes before bringing down the cone. As the systems exhibited a non Newtonian flow, the intrinsic viscosity was measured by using the non-newtonian equation which is Log G= N Log (S – F) – Log n. Whereas, G is the shear rate in sec^{-1} , S is the shear stress in Pascal, F is the yield value, n is the viscosity and N is the slope of log (S-F) against log G plot. The intrinsic viscosity of the freshly prepared formulation was measured and was used to compare the change in these measurement values after specified time intervals at different temperatures studied.

Drug content measurement

Drug content in formula E16 was analysed by using the validated HPLC method. In this study, 1 gm of the nano-cream was dissolved in the mobile phase (acetonitrile: methanol:5mM disodium hydrogen phosphate:glacial acetic acid). The samples were diluted with the mobile phase and filtered before drug content was determined by the modified HPLC method.

Statistical Analysis

Statistical analysis was performed by using the one-way ANOVA test to determine difference of all the parameters studied at the initial and after 90 days of observation and at all storage conditions. Statistically, a significant difference was considered at a p value of less than 0.05.

Results and discussion

The compositions of POEs as the oil phase, phosphate buffer as the aqueous phase and Tween 80:Span 20 (80:20) with HLB value of 13.72 as the emulsifier were at the ratio of 37:25:38 respectively. The formulae E14, E15 and E16 contain phosphate buffer of pH 4, pH 6 and pH 7.4 respectively.

A preliminary study was done at an accelerated temperature of 40°C to test the physical appearance of the samples. The study revealed that nano-cream formulae containing piroxicam were highly sensitive to the pH of the external phase of tested nano-creams. Presence of the weak acidic piroxicam at the interface makes it easy to diffuse to the external phase where it is highly affected by the pH of the phase. There was precipitation of the drug from formulations E14 and E15 but not from formulation E16. The reason behind the precipitation may be due to the pH based difference in the solubility of piroxicam. The higher the pH of the external phase of the cream, the greater is the solubility of piroxicam [8]. Formulation E16 (pH 7.4) posses the highest solubility of piroxicam and no precipitation occurred. So, further stability studies were carried out only on formulation E16.

Particle size measurement

The results shown in table 1 show that the droplets size of the nano-cream formula which was stored at different temperatures and measured at different time intervals are not significantly different from the initial size ($p > 0.05$). These results indicate that aggregation and coalescence of the dispersed droplets did not take place significantly. Stability of the system can be further demonstrated by the minimal increase in droplet size.

Table 1. Droplet size measurement in nm of formulation E16 subjected to stability testing at different temperatures for specified time intervals. All data are presented as mean±SD, (n=3).

Storage temperature	Droplets size measurement after specified days of storage					
	0	15	30	45	60	90
4 °C	132.39± 1.69	133.67± 1.09	134.37± 1.56	133.73± 1.87	134.35± 0.40	134.13± 1.63
25 °C	132.39± 1.69	133.18± 0.39	133.51± 0.85	133.95± 1.51	134.74± 0.76	134.60± 1.15
40 °C	132.39± 1.69	134.84± 1.06	133.21± 1.92	134.6±0 .97	135.25± 0.63	135.73± 1.56

Insignificant increase in particle size measurement under the accelerated study can be correlated to least free energy of the system that makes the system least susceptible to the effect of Ostwald ripening and coalescence. The reason behind this stabilization effect may be attributed to the high surfactant concentration in the prepared nano-cream. As concentration of the surfactant increases in the emulsion, there is also an increase in the film thickness that is formed around the dispersed phase droplet by the surfactant molecules, thereby leading to an increased steric hindrance which prevents coalescence [9].

It has been reported by Desmet *et al.* (1999) and Liu *et al.* (2006), that there was a decrease in the ripening rate as the amount of surfactant increased. It has been postulated that if oil is solubilized in surfactant micelles, it can be enclosed within the micellar system and is not dispersed directly in the continuous phase, thus it is not subjected to the same mass

transfer between the droplets as when they are outside the micellar system. In these systems, the oil is withdrawn from the continuous water phase into the micelles, thus causing the ripening rate to decrease due to the low solubility of the oil in water. Similar results were observed by Ahmad *et al*. (1996) for emulsion containing palm oil.

Table 2. Conductivity measurements in Ms of formulation E16 subjected to stability testing at different temperatures for specified time intervals. All data are presented as mean±SD, (n=3).

Storage Temperature	Conductivity measurement after specified days of storage					
	0	15	30	45	60	90
4°C	349±2.31	350±3.05	348±1.15	348±0.57	349±2.08	348±2.00
25°C	349±2.31	349±2.08	347±2.08	346±0.57	347±1.53	347±1.15
40°C	349±2.31	347±0.57	347±1.00	346±0.57	347±0.57	345±1.00

Conductivity measurement

Table 2 reveals that the initial conductivity of formulation E16 was 349±2.31 µs. After three months of the stability study at 40°C, 25°C and 4°C, the conductivity values were read as 345±1.0, 347±1.15 and 348±2.00 µs respectively. It was found that there was no significant change ($p > 0.05$) in the conductivity measurements for 3 months storage at the temperatures studied. Insignificant change in the conductivity measurements at the bottom of the container indicates that the bottom of the container continues to contain same amount of oil phase within the time frame of the stability study. Accordingly, separation and upward movement of the oil phase is concerned with the increase in the conductivity at the bottom of the emulsion container due to lowered or reduced interference from the lower number of the oil droplets. The non significant change in conductivity could be used to indicate that there was no creaming or sedimentation in the cream during the period of the study. This stabilization effect may be attributed to the close density of the dispersed phase (POEs) and the dispersion medium (phosphate buffer of pH 7.4) which are 0.8553 gm/cm^3 and 0.997 gm/cm^3 respectively [11]. Moreover, small droplets sizes of dispersion phase and high viscosity of the preparation are considered as stabilization factors [12].

pH measurement

From table 3, it is evident that pH of the fresh preparation of formula E16 was 7.06±0.05. After three months of storage at 40°C, 25°C as well as 4°C, the pH measurements were found to be 6.90±0.03, 6.91±0.06 and 6.97±0.1 respectively. It is apparent from table 3 that the pH of the nano-cream did not change significantly during the period of stability study at all conditions of the study. This could be used as an indication that ionization and hydrolysis had not taken place significantly during the storage period of the study.

Table 3. pH Measurement of Formulation E16 subjected to stability testing at different temperatures for specified time intervals. All data are presented as mean±SD, (n=3).

Storage Temperature	pH measurement after specified days of storage					
	0	15	30	45	60	90
4°C	7.06±0.05	7.05±0.07	7.05±0.04	7.04±0.04	7.03±0.02	6.97±0.10
25°C	7.06±0.05	7.04±0.03	7.06±0.05	7.02±0.02	6.96±0.07	6.91±0.06
40°C	7.06±0.05	7.06±0.04	7.02±0.07	6.92±0.02	6.94±0.04	6.90±0.03

Viscosity Measurement

As shown in tables 4 it is evident that the rheological characteristics of the sample were not significantly affected ($p > 0.05$) by the storage conditions after three months of observation. Relative viscosity of the sample measured did not change significantly during the time of the stability study. This may be due to the intactness of hydrogen bonds between the polyoxyethylene chains of the surfactants within the time frame of stability study. The surfactants from the Tween series have 20 polyoxyethylene groups, and their dispersion in water is due to the hydrogen bonds between water molecules and these polyoxyethylene groups [13].

Table 4. Relative viscosity measurement in poise of formulation E16 subjected to stability testing at different temperatures for specified time intervals. All data are presented as mean±SD, (n=3).

Storage Temperature	Relative viscosity measurement after specified days of storage					
	0	15	30	45	60	90
4°C	14802.67±61.20	14774±37.64	14728.30±36.12	14713±35.04	14671.67±53.41	14695±113.64
25°C	14802.67±61.20	14756.67±35.01	14712.33±58.62	14658.67±111.10	14609.33±28.57	14566.67±186.40
40°C	14802.67±61.20	14770±74.65	14741.67±42.15	14708±24.64	14615±49.57	14568.33±169.80

Viscosity changing of nano-creams emulsified by polyoxyethylene non-ionic surfactants is related to the stability of the network built by their polyoxyethylene chains. As these surfactants are stable under various conditions, no change of viscosity is suspected. These results are in line with the reported cloud point of Tween 80 which is about 70^0C [14], thus no rheological changes would occur when the stability study was only conducted at temperature 40°C and below. These results also reflect that there was minimal water evaporation, thereby rendering the stability of the emulsion. Similar results were observed for celecoxib nano-emulsion which was stabilized with only Tween 80 [15] indicating that non-ionic surfactants namely Tween 80 and Span 20 and similar class of surfactant is suitable for producing stable nano-cream.

Table 5. Drug content measurement in percentage of formulation E16 subjected to stability testing at different temperatures for specified time intervals. All data are presented as mean±SD, (n=3).

Temperature	Drug content measurement after specified days of storage					
	0	15	30	45	60	90
4°C	100.01±0.01	100.01±0.015	99.94±0.03	99.86±0.20	99.70±0.14	99.36±0.28
25°C	100.01±0.01	99.96±0.07	99.83±0.08	99.28±0.15	99.05±0.18	98.62±1.08
40°C	100.01±0.01	99.88±0.05	99.08±0.24	98.67±0.16	98.17±0.15	98.8±0.96

Drug content measurement

The drug content in the sample was within the range of 99-101% of the original drug content. The samples were diluted with the mobile phase and filtered before drug content was determined by the modified HPLC method. The stability study at different temperatures exhibited that the pH and drug content of the E 16 formula was almost constant for the whole duration of the study.

Conclusion

The prepared 0.5% piroxicam nano-cream which contains 37:25:38 of (0.2M phosphate buffer pH 7.4:POEs:surfactant mixture of Tween 80 and Span 20 (80:20) was stable against creaming, coalescence, phase separation and Ostwald ripening. It can be concluded that weak acidic drugs with low solubility in the aqueous as well as the oil phase is highly affected by the pH of the external phase. Furthermore, using of non-ionic surfactants combination can produce a highly stable nano-cream preparation.

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